PCR DETECTION OF *Nosema apis* /*Nosema ceranae* SPORES IN HONEY SAMPLES

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INTRODUCTION

Nosemosis is the most widespread of adult bee diseases and causes significant economic losses to beekeepers worldwide.

The availability of *Nosema apis/Nosema ceranae* DNA detection protocol from different hive products (i.e. honey, pollen, wax) could contribute to the systematic health status control of bee hive. Its application could focus on the early detection of infection before the clinical onset and consequently the early application of prophylactic and control measures. Moreover, it could allow monitoring the spread of infection in a given region and potentially identify the presence of nosemosis risk.

AIM OF THE STUDY

The present study describes the development of a PCR protocol for the detection of *Nosema apis/Nosema ceranae* spores in honey samples.

MATERIAL AND METHODS

Honey samples were submitted to the Istituto Zooprofilattico Sperimentale delle Venezie for monitoring the quality of honey production in Veneto region. DNA extraction and PCR assay were established using a *Nosema* spp. negative honey sample aliquoted in duplicate and inoculated with *N. ceranae* spores. 20 ml of honey were incubated at 50°C for about 20 minutes and then 1 ml was mixed with equal volume of phosphate buffer saline (PBS). After centrifugation for 30 minutes at 3000 x g, the supernatant was discarded and the pellet was washed with 1.5 ml of PBS and further centrifuged for 10 minutes at 10000 x g. The pellet was subjected to microscopic observation for *Nosema* spp. spores and to DNA extraction by using QIAamp DNA Mini Kit with a pre-incubation with chitinase or lysozyme. DNA was subjected to PCR and sequencing (Higes *et al* 2006). PCR products were analysed on 7% acrylamide gels and visualized after silver staining.

In order to evaluate the sensibility of the protocol, 1 ml of negative honey was inoculated with *N. ceranae* spores in different concentrations (from $1x10^6$ to $1x10^1$ spores/ml honey). The samples were prepared in triplicates (a, b, c: pre-incubated with lysozyme; a', b', c': pre-incubated with chitinase) and tested in two successive days.

To investigate the practical value of this assay, 30 commercial honey samples and 8 honey samples from brood comb were analysed. 20 ml of the above honey samples were incubated at 50°C for about 20 minutes and were shaken for better distribution of *Nosema* spores, when present.

RESULTS

DNA extraction and PCR assay were established using a *Nosema* spp. negative honey sample inoculated with *N. ceranae* spores. A 250 bp PCR product for the 16S rRNA gene of *Nosema* spp. was obtained. The protocol showed high sensitivity and reproducibility, since it detected up to $0.5x10^2$ spores/ml of *N. ceranae*. Comparable results were obtained by using either chitinase or lysozyme pre-incubation (Figure 1). No PCR product was observed in all 30 commercial honey samples tested. Of 8 brood comb honey samples (BH), 5 were negative and, after PCR product sequencing, 2 were positive for *N. ceranae* and in one both *N. apis*/*N. ceranae* were present (Table 1 and Figure 2).

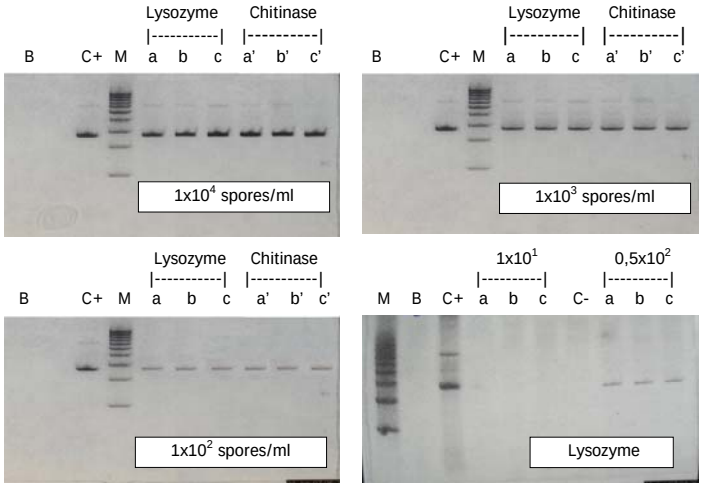

Figure 1. Specific PCR products from inoculated honey samples with concentrations from $1x10^4$ to $1x10^1$ of *N. ceranae* spores/ml honey. A 250 bp specific PCR product was detected up to $0.5x10^2$ spores/ml honey.
[B: blank; M: DNA ladder 100 bp; C+: positive control; C-: negative control]

Sample	*Nosema apis*	*Nosema ceranae*
1BH	Positive	Positive
2BH	Negative	Positive
3BH	Negative	Positive
4BH	Negative	Negative
5BH	Negative	Negative
6BH	Negative	Negative
7BH	Negative	Negative
8BH	Negative	Negative

Table 1. Summary of PCR results of 8 Brood comb Honey samples (BH) for *N. apis*/*N. ceranae* spores.

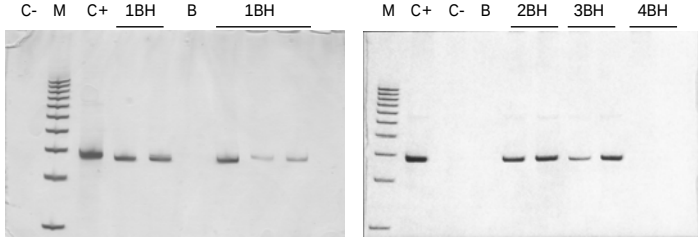

Figure 2. PCR results of Brood comb Honey samples (BH) for *Nosema* spp.
1BH: positive for *N. apis* and *N. ceranae* (confirmed by sequencing)
2BH, 3BH: positive for *N. ceranae* (confirmed by sequencing)
4BH : negative sample
[B:blank; M: DNA ladder 100 bp; C+: positive control; C-: negative control]

DISCUSSION

The established protocol for *Nosema* spp. detection in honey demonstrated high sensitivity and reproducibility, but also proved to be time consuming and labour intensive. Its application on bee hive products other than honey, such as wax and pollen, might be useful for screening bee hive health status for *N. apis/N. ceranae*, even at the absence of clinical signs.

References

1. Higes M, Martin R, Meana A. 2006. *Nosema ceranae*, a new microsporidian microsporidian parasite in honeybees in Europe. J Invertebr Pathol 92:81-83.

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